Various forms of recombinant antibodies

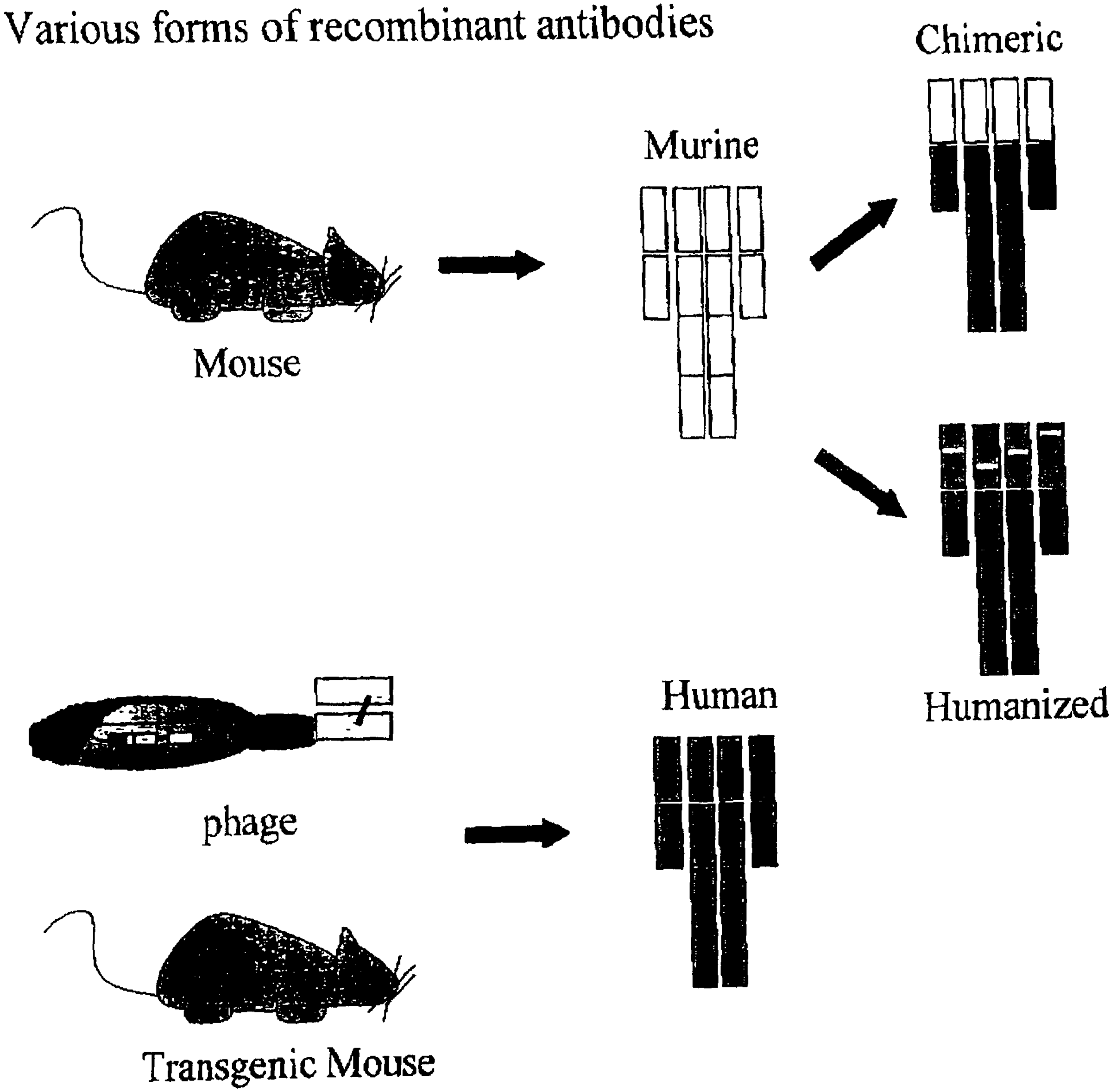


Figure 1

Steps in flowcytometry-based selection of phage binding to the cell surface of subpopulations of cells

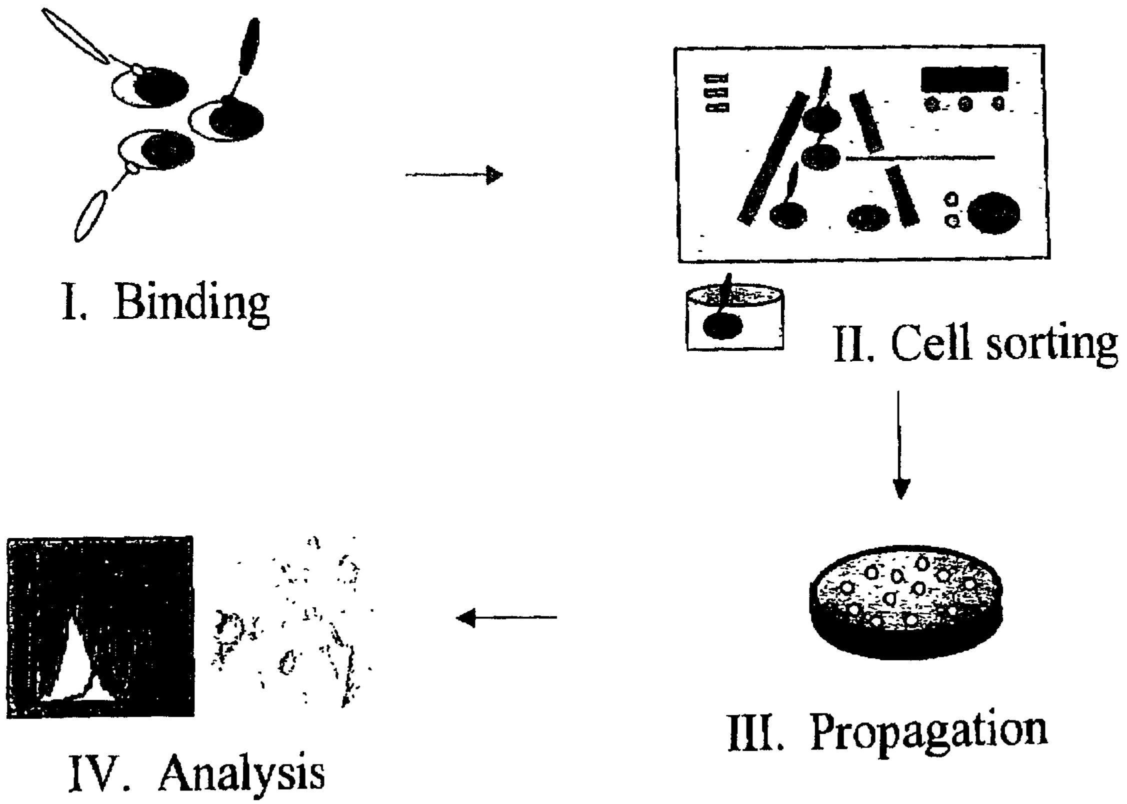


Figure 2

Sort criteria for the isolation of multiple myeloma cells
based on CD38 expression
and forward/side scatter pattern

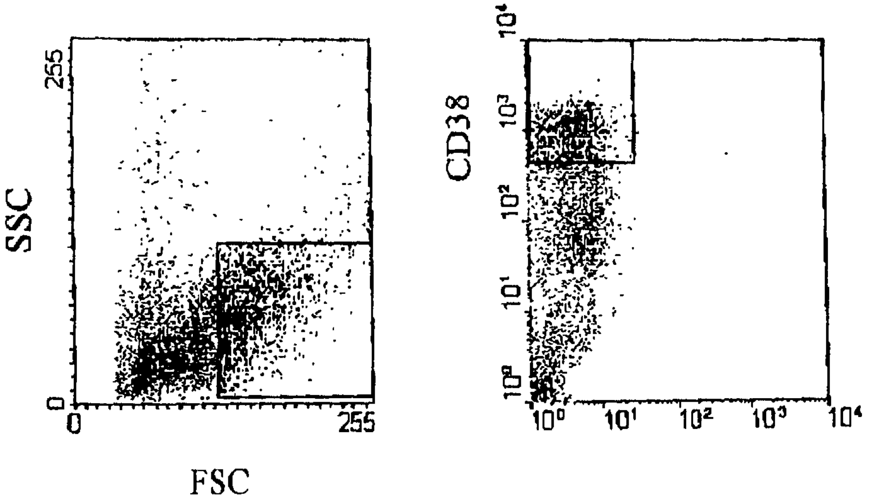


Figure 3

Staining of blood, spleen, tonsil, multiple myeloma (MMBM) and adult bone marrow (ABM) with a control phage (upper row) or with K53 (lower row).

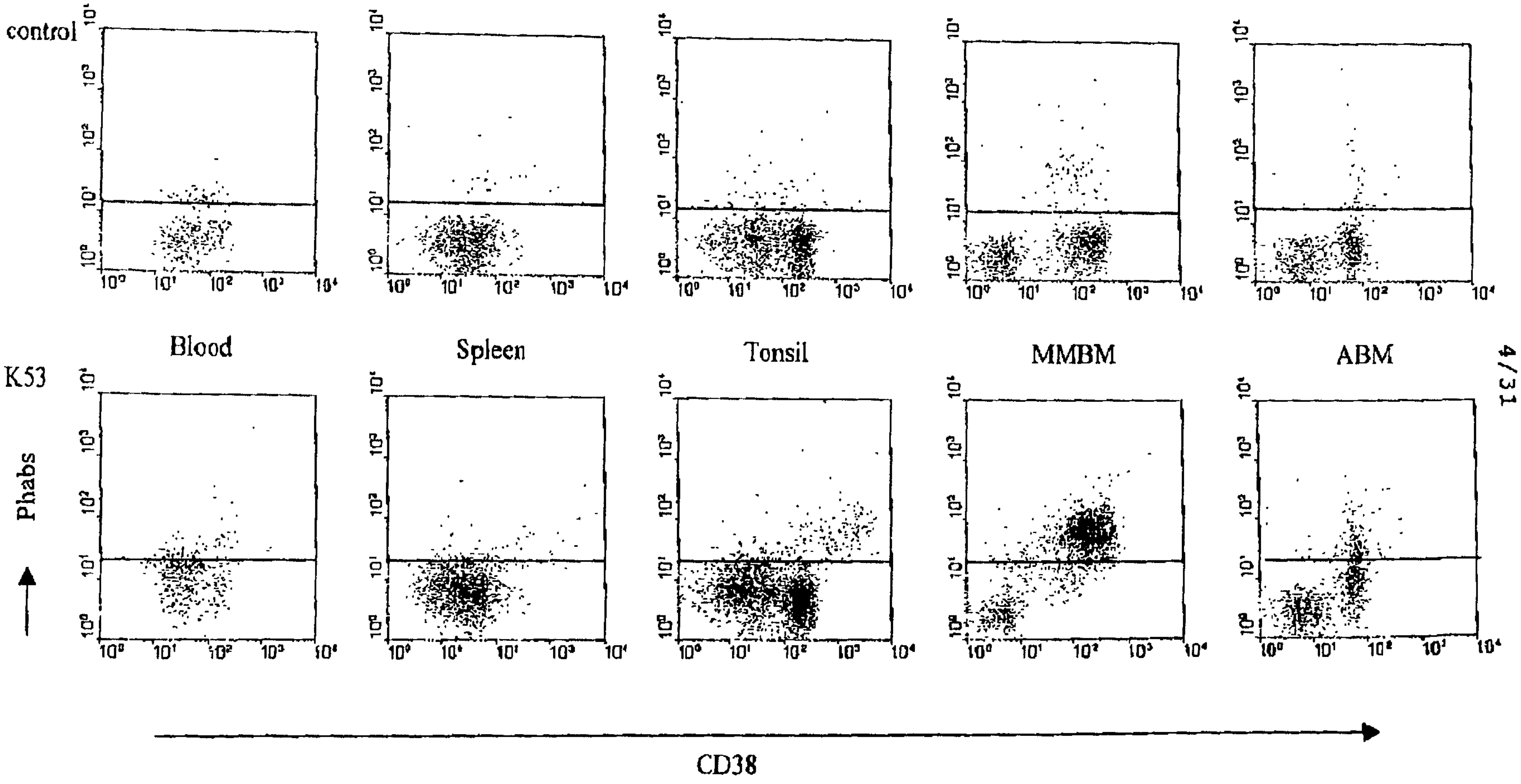


Figure 4

VH and VL gene utilization and deduced amino acid sequence of the CDR3 regions of K19, K29 and K53

scFv	CDR3	VH*	VL
K19:	DYRYPSTSWFDS	DP31	Vκ3
K29:	ARRWAAFDY	DP44/45	Vκ2
K53:	GMMRGVFDY	DP14	Vκ2

Figure 5

Staining of Bone marrow of a MM patient with K53 (left panel) and staining of sorted K53+ cells with May Grunwald Giemsa.

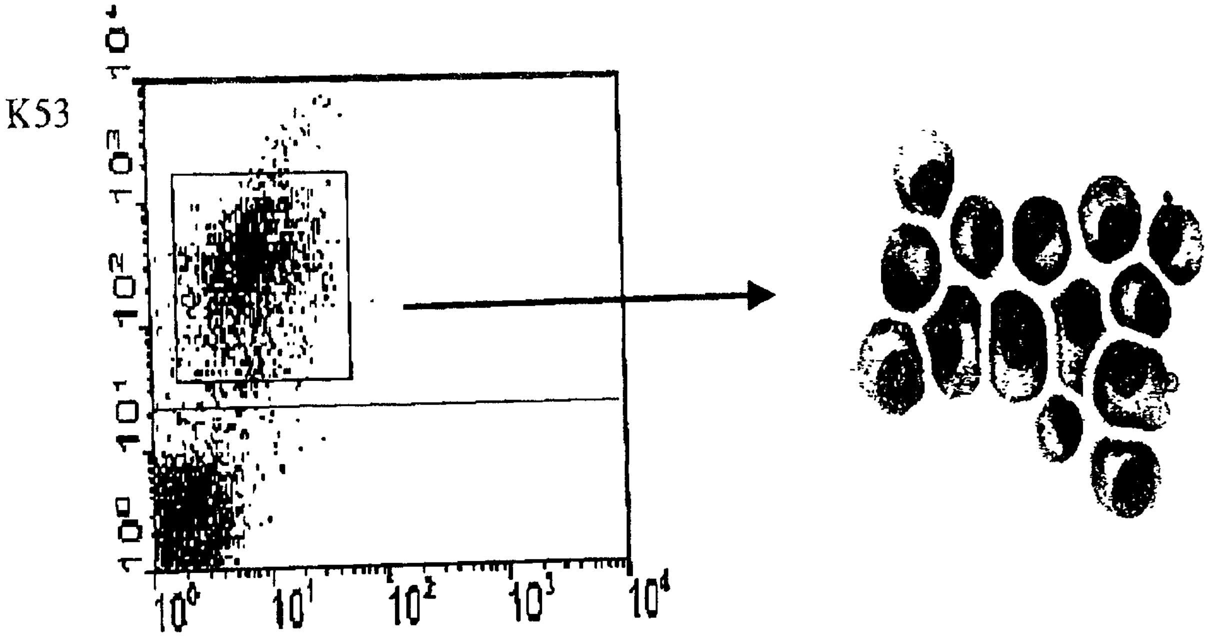


Figure 6

Staining of insect cells transfected with a human placenta cDNA library with K19 antibody after subsequent rounds of flowcytometric isolation

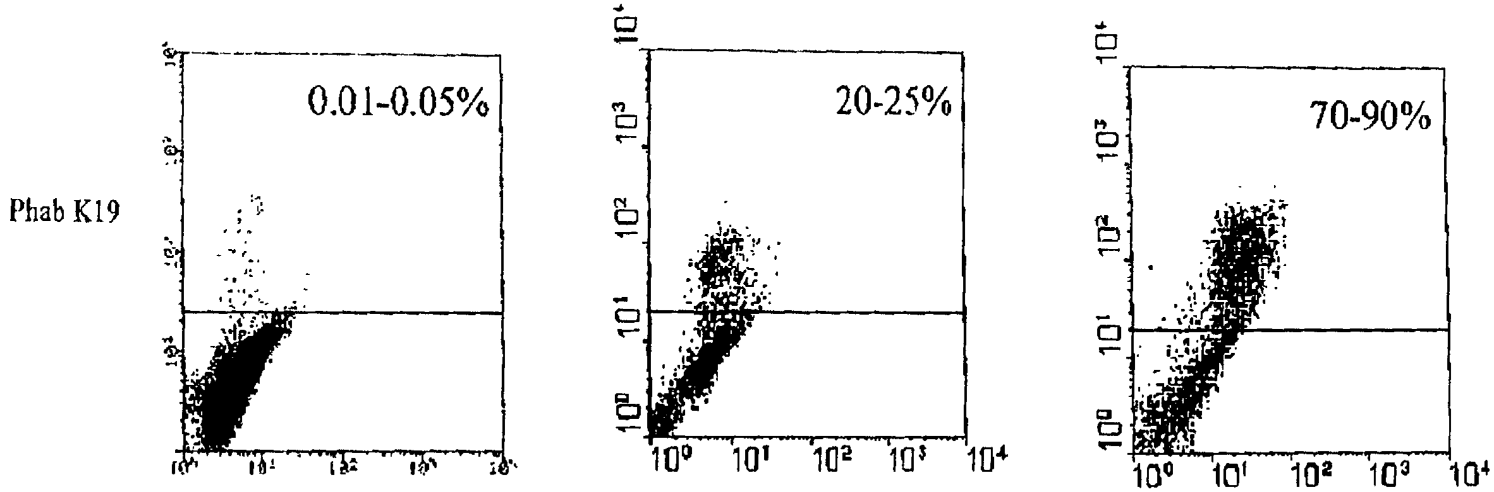


Figure 7

Human monoclonal antibodies K19 and K53 detect glycosylation variants of CD46

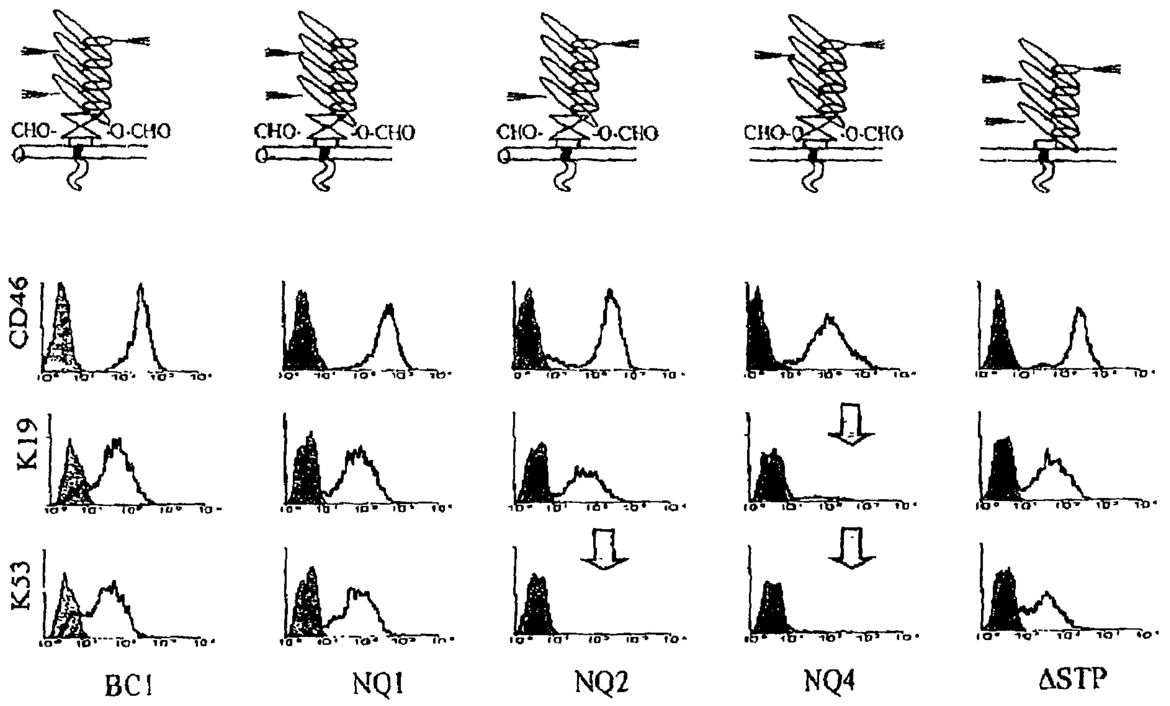


Figure 8

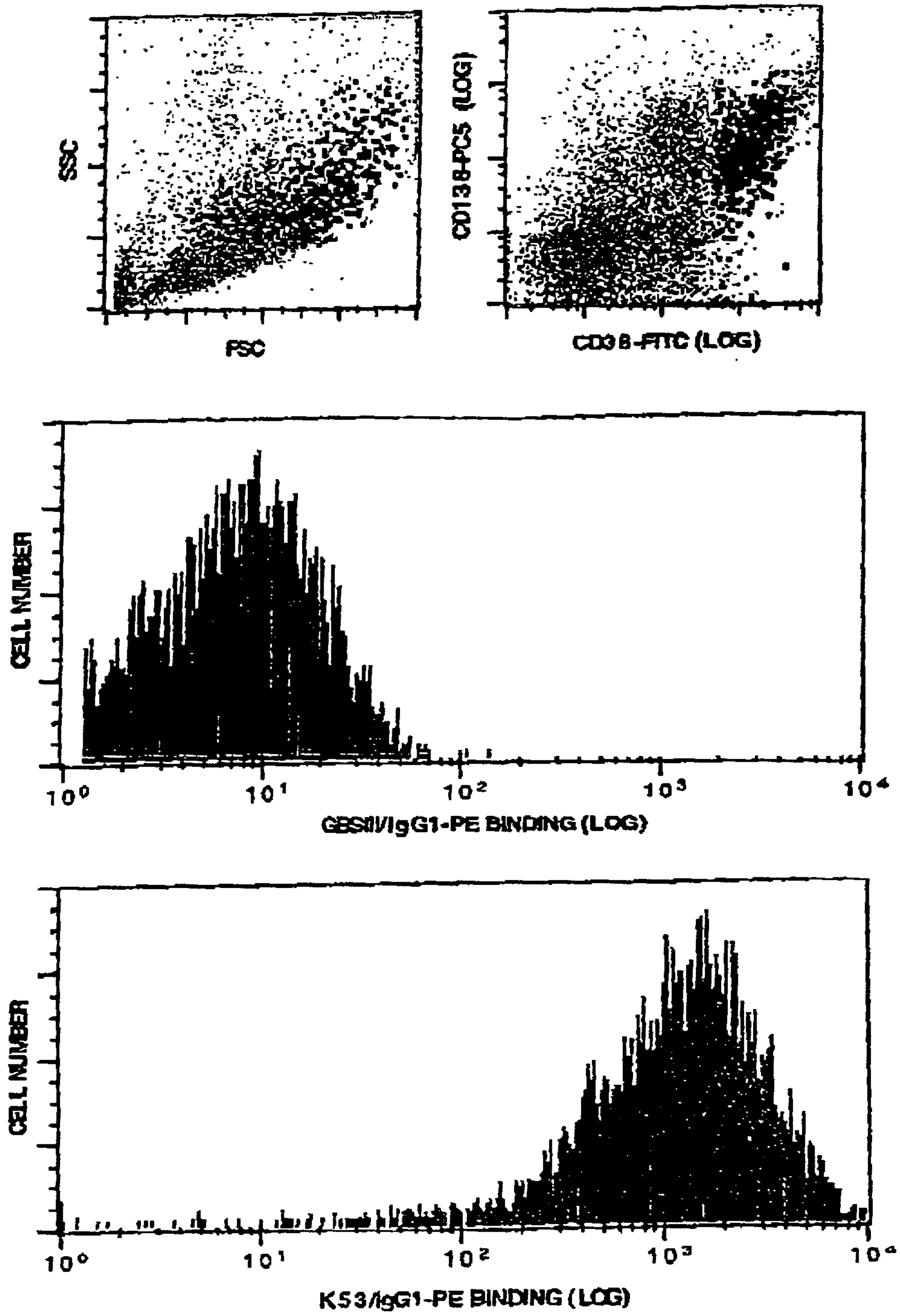

SSC
FSC
CD138-PC5 (LOG)
CD38-FITC (LOG)
CELL NUMBER
10^0
10^1
10^2
10^3
10^4
GBSIII/IgG1-PE BINDING (LOG)
CELL NUMBER
10^0
10^1
10^2
10^3
10^4
K53/IgG1-PE BINDING (LOG)

FIGURE 9

Tumor cell killing with with human monoclonal antibodies using a xenograft model of colon carcinoma

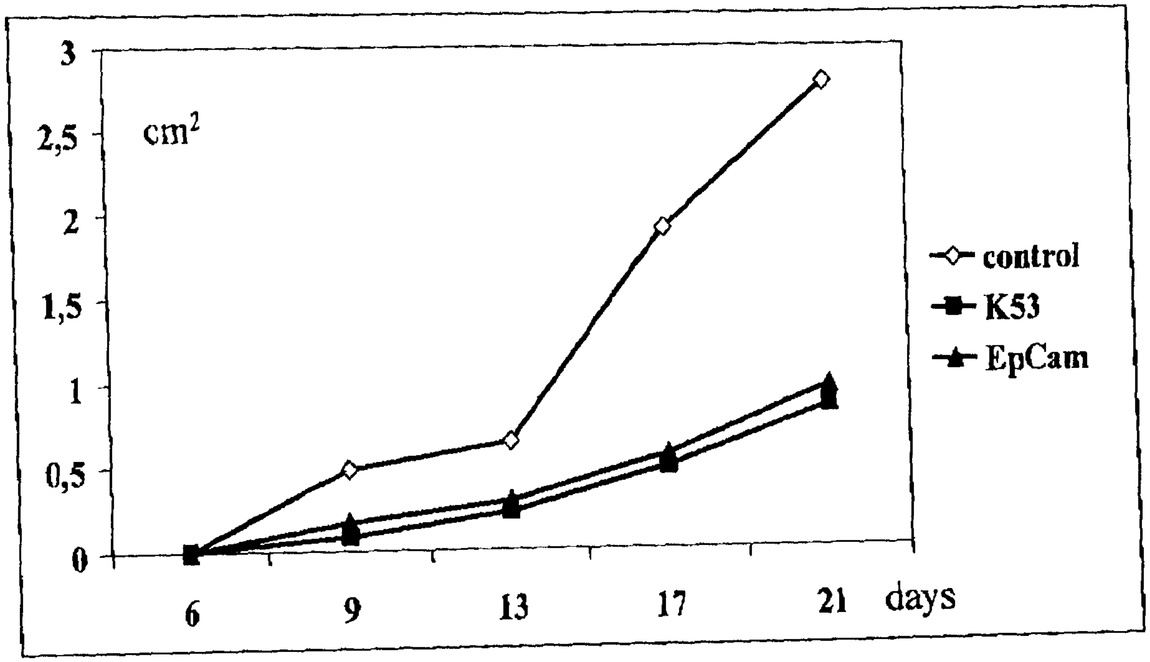
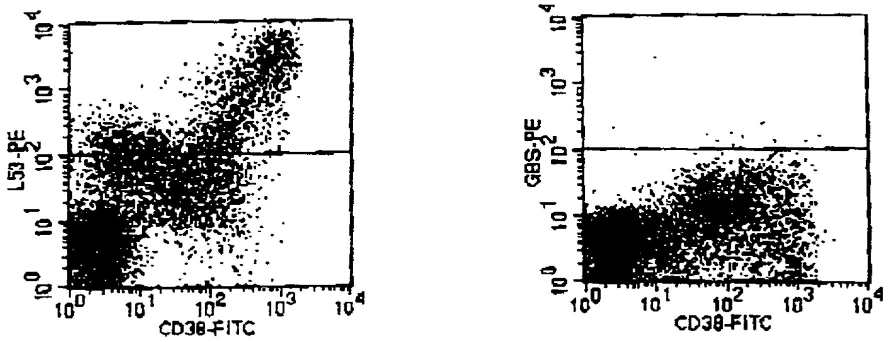


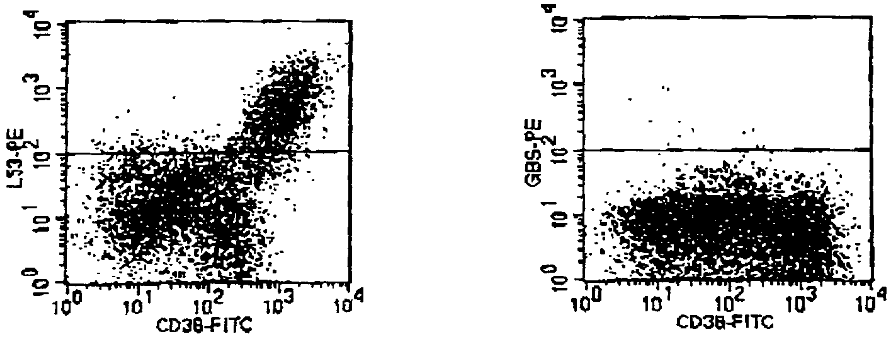
Figure 10

STAINING BONE MARROW OF MULTIPLE MYELOMA PATIENTS
PRIMARY TUMORS

P1 (FRESH)



P2 (N2)



P4 (N2)

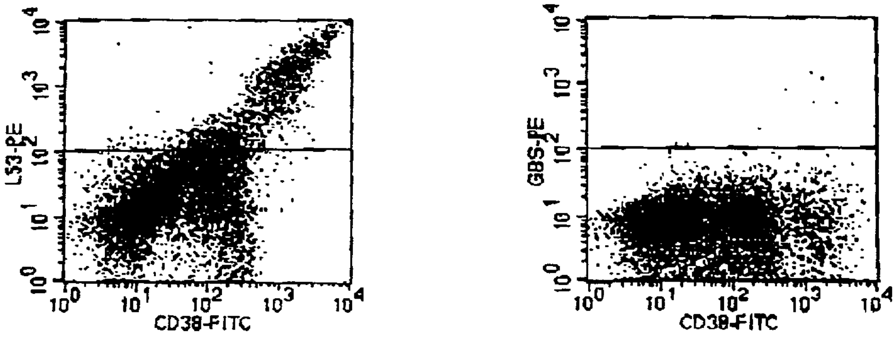


Figure 11A

STAINING BONE MARROW OF MULTIPLE MYELOMA PATIENTS RESPONSIVE TUMORS

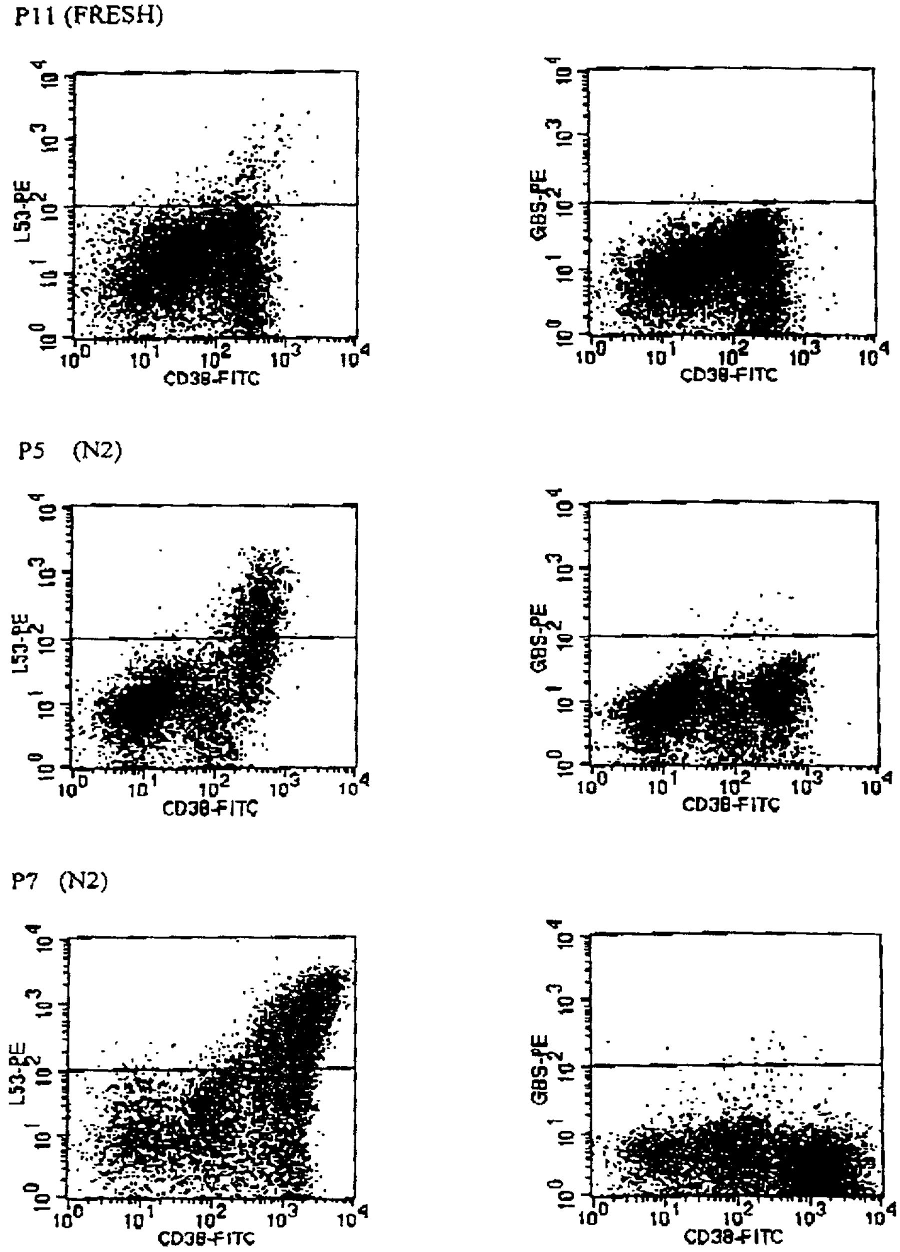
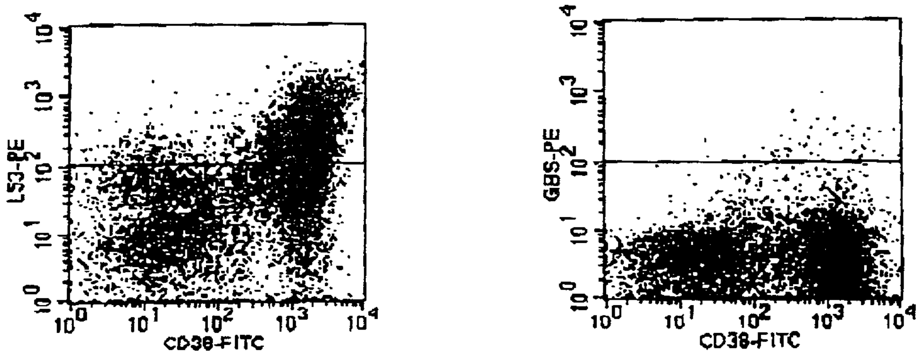


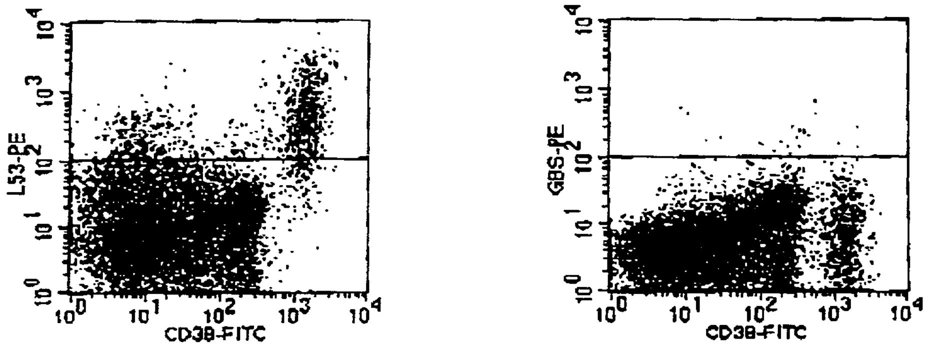
Figure 11B

STAINING BONE MARROW OF MULTIPLE MYELOMA PATIENTS REFRACTORY TUMORS

P8 (FRESH)



P10 (FRESH)



P13 (FRESH)

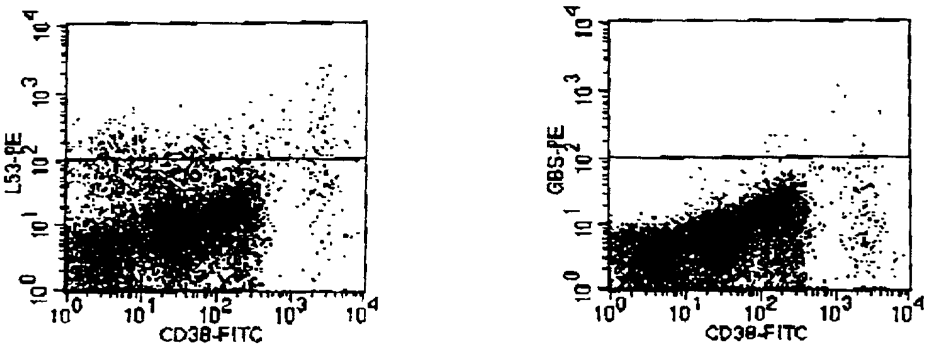


Figure 11C

STAINING BONE MARROW OF MULTIPLE MYELOMA PATIENTS REFRACTORY TUMORS

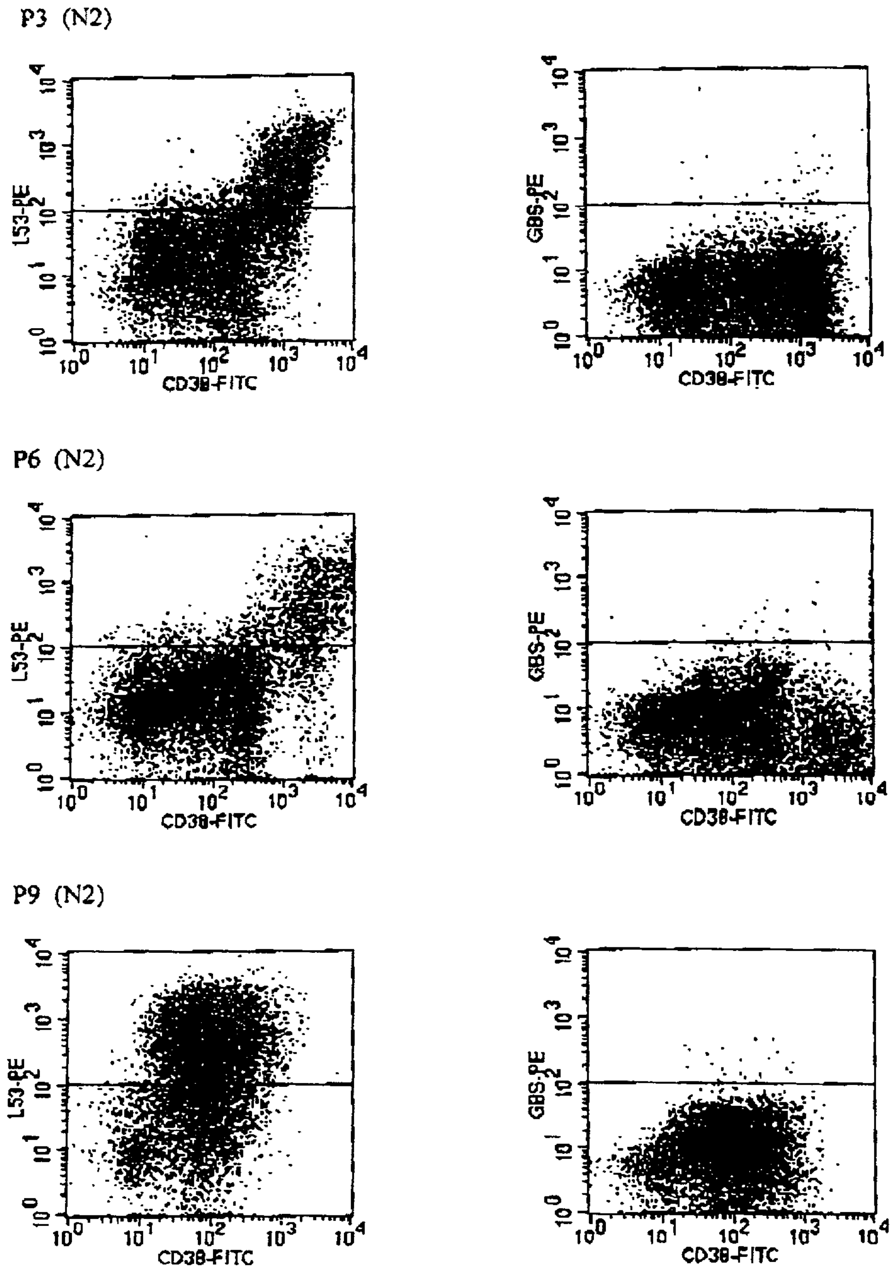
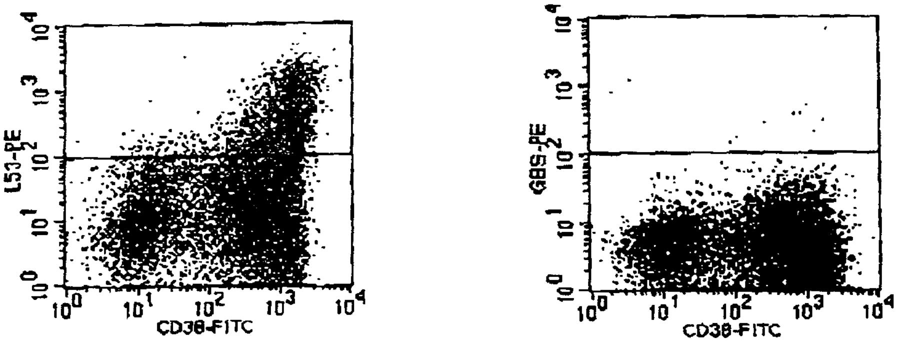


Figure 11D

STAINING BONE MARROW OF MULTIPLE MYELOMA PATIENTS REFRACTORY TUMORS

P12 (N2)



P8 (N2)
(FROM ABOUT ONE YEAR BEFORE THE FRESH MATERIAL)

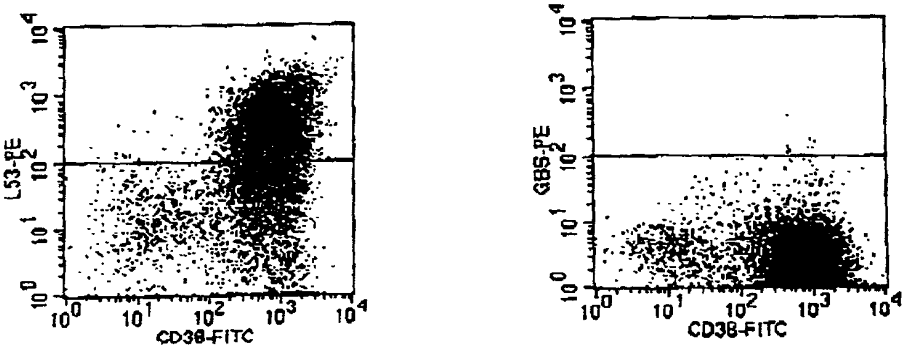
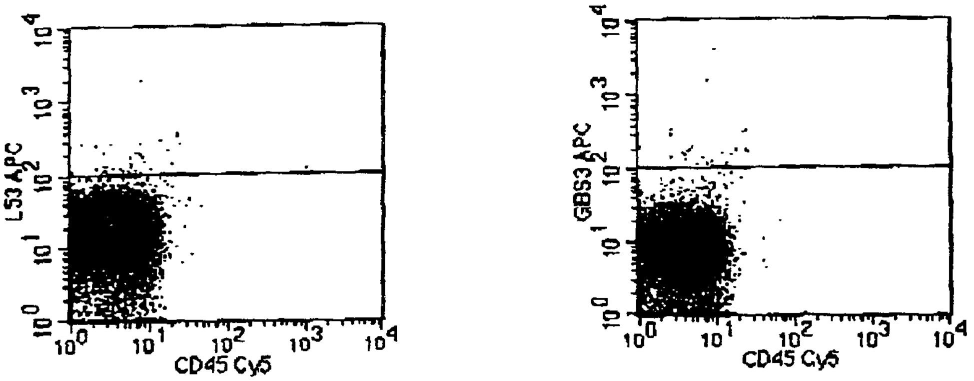


Figure 11E

STAINING BONE MARROW OF PATIENTS WITH OTHER LEUKEMIC TUMORS

A1 (T-ALL)

GATE FOR TUMOR CELLS : CD45-



A9 (B-NHL)

GATE FOR TUMOR CELLS : CD20+

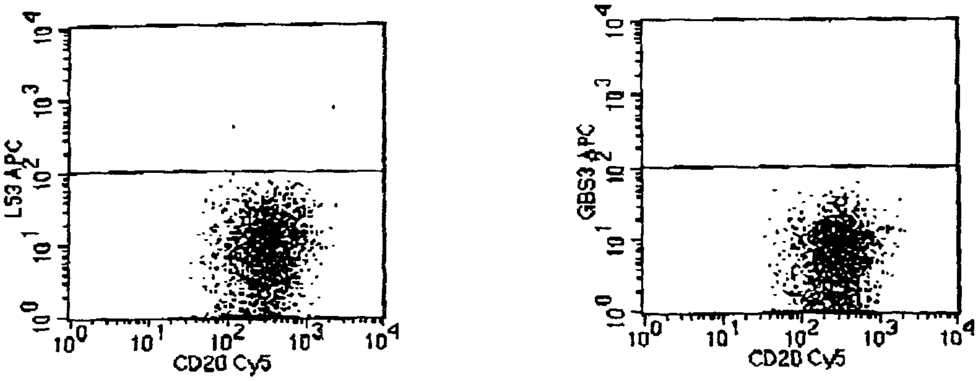
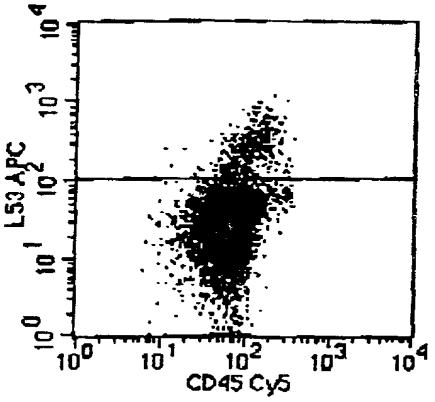


Figure 12A

STAINING BONE MARROW OF PATIENTS WITH OTHER LEUKEMIC TUMORS

A4 (M0/M1 MYELOID)

GATE FOR TUMORCELLS : CD45 WEAK



A5 (M4 MYELOID)

GATE FOR TUMORCELLS : CD45+

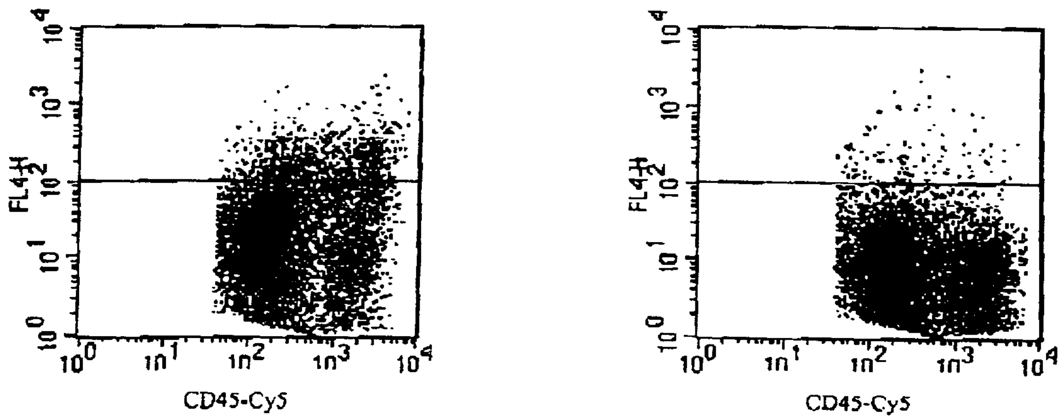


Figure 12B

STAINING BONE MARROW OF PATIENTS WITH OTHER LEUKEMIC TUMORS

A7 (CLL)

GATE FOR TUMOR CELLS : CD19+

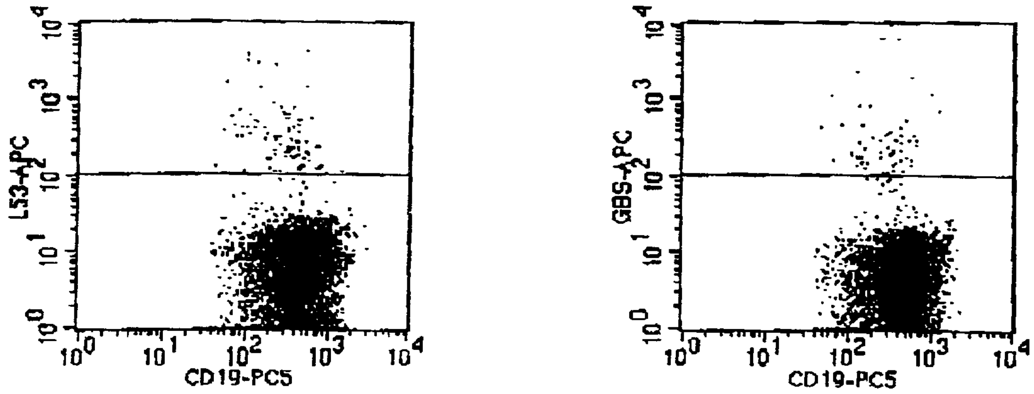
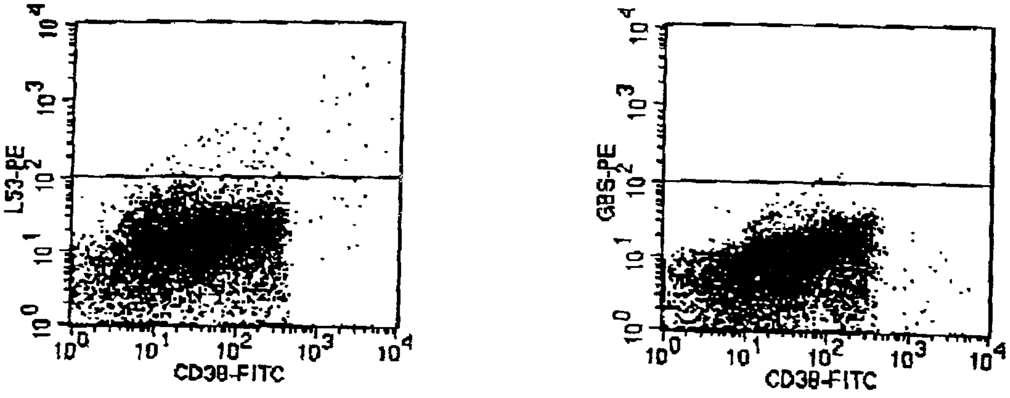


Figure 12C

STAINING OF NORMAL TISSUES

NORMAL BONE MARROW



TONSIL

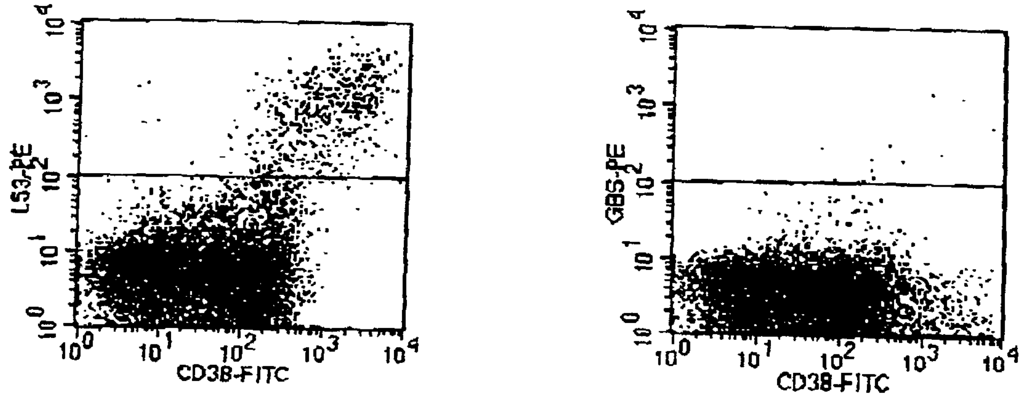


Figure 13

STAINING OF NORMAL BLOOD

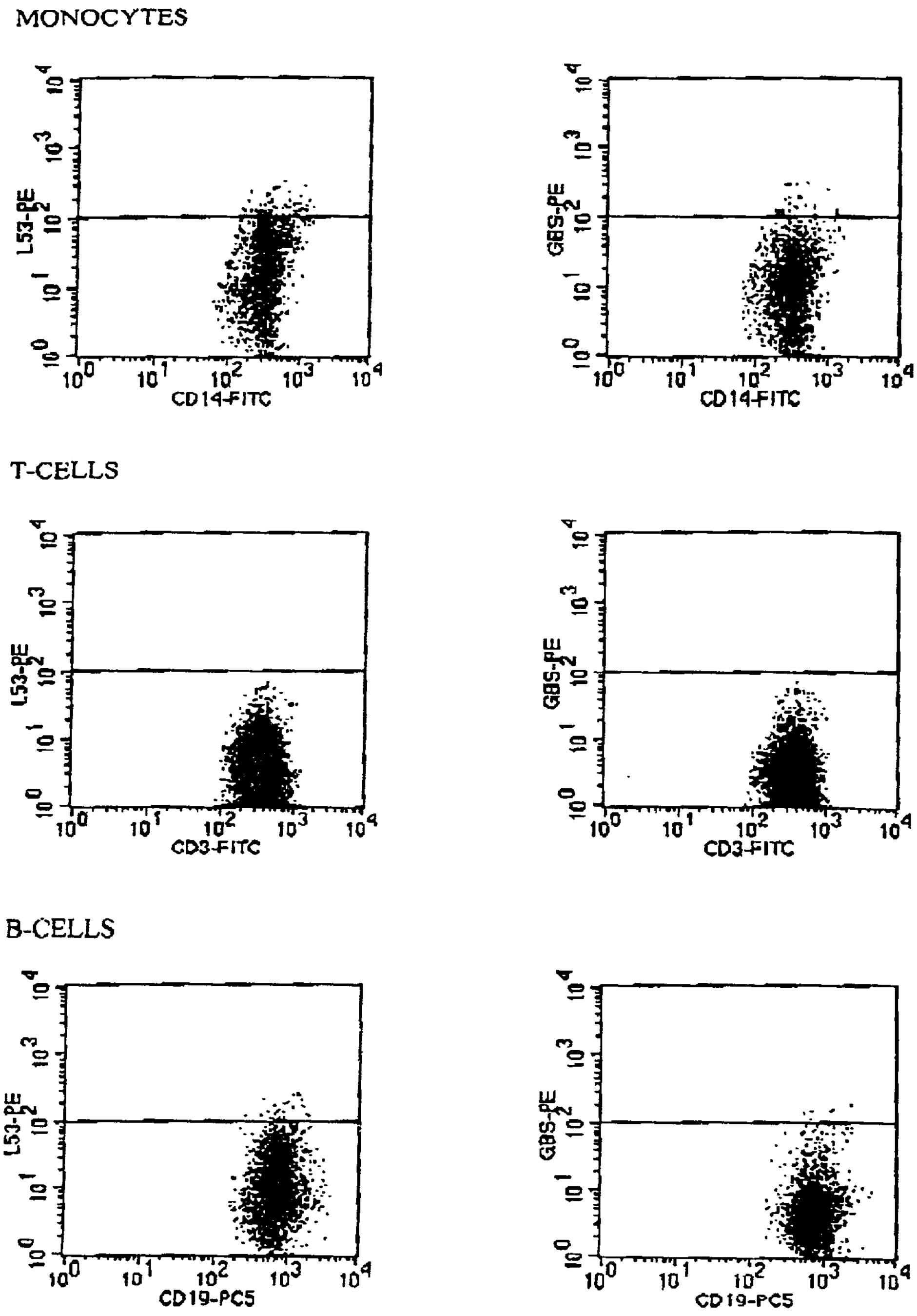
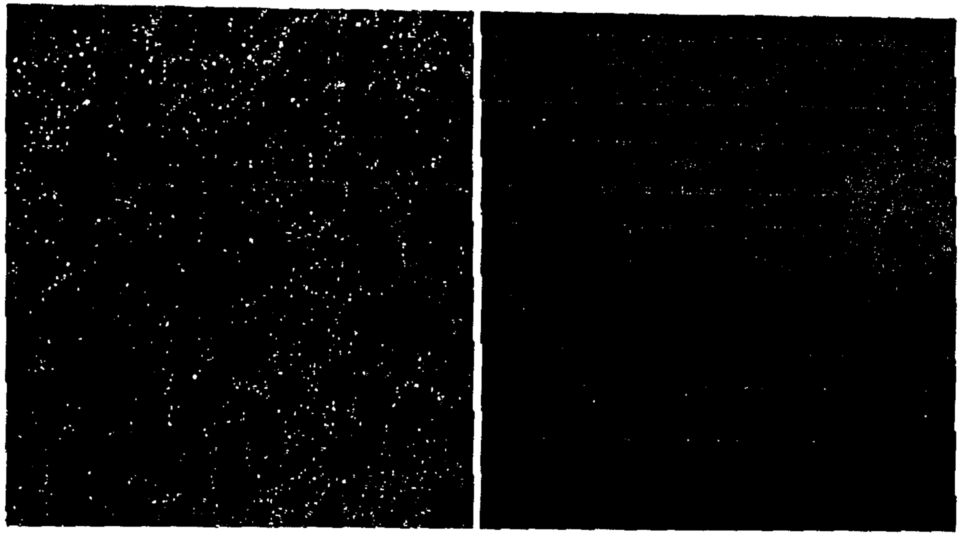


Figure 14

Specific staining of human colon tumor tissue with Anti-CD46 monoclonal antibody K53/IgG1



K53/IgG1 GBS III

Figure 15

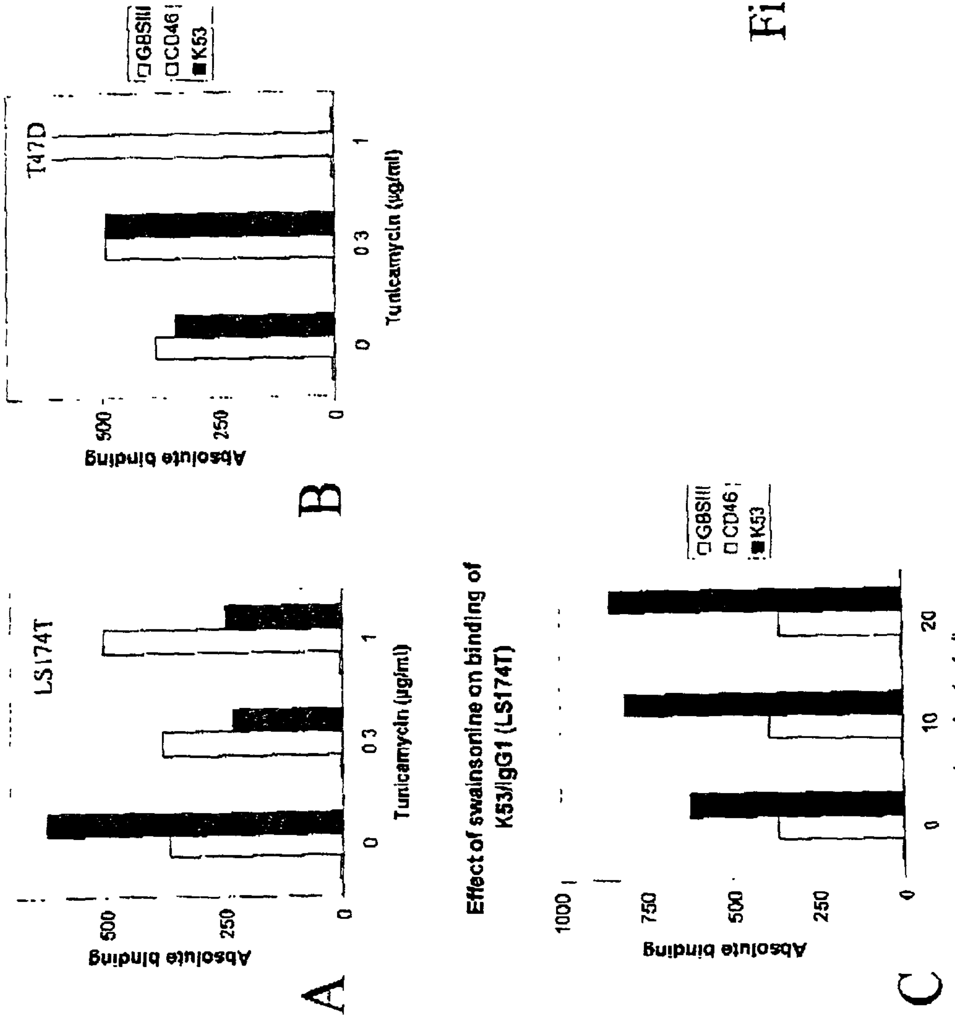

A
LS174T
Absolute binding
500
250
0
0
0.3
1
Tunicamycin (µg/ml)
B
T47D
Absolute binding
500
250
0
0
0.3
1
Tunicamycin (µg/ml)
☐ GBSIII
☐ CD46
■ K53
C
Effect of swainsonine on binding of K53/IgG1 (LS174T)
Absolute binding
1000
750
500
250
0
0
10
20
Swainsonine (µg/ml)
☐ GBSIII
☐ CD46
■ K53

Figure 16

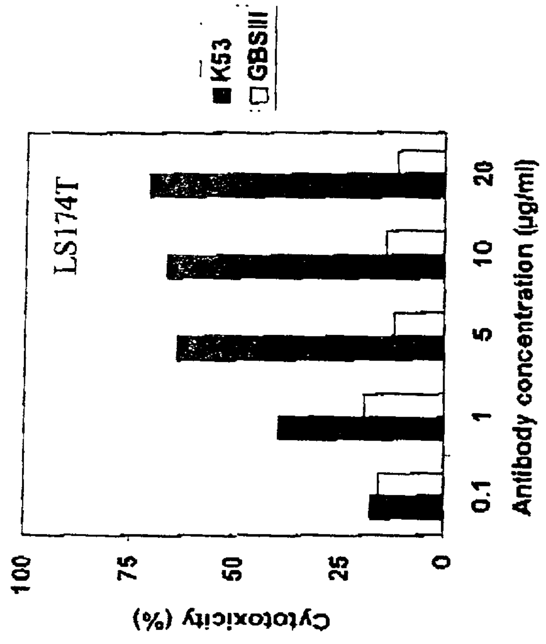

LS174T
■ K53
□ GBSIII
Cytotoxicity (%)
100
75
50
25
0
0.1
1
5
10
20
Antibody concentration (µg/ml)

Figure 17

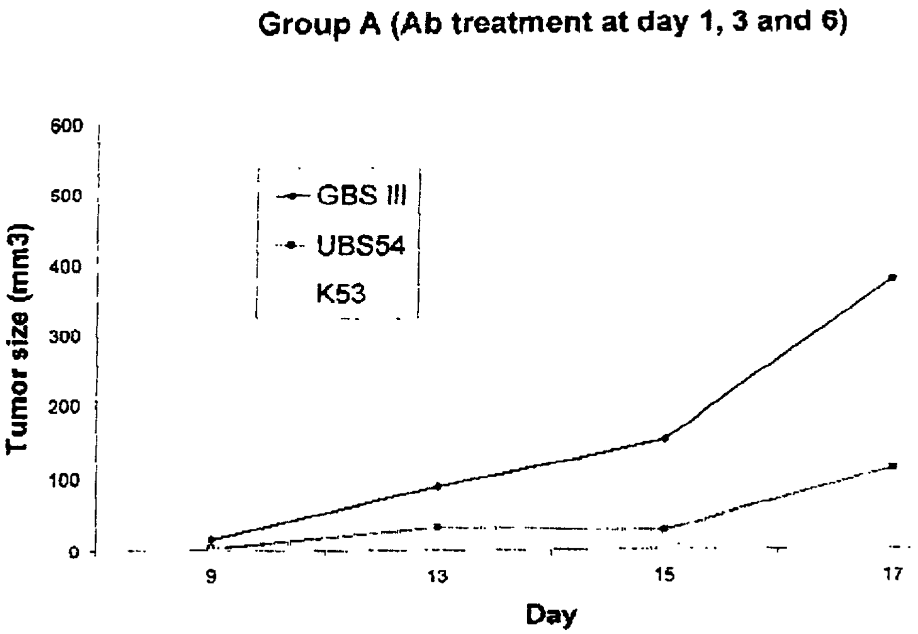


Figure 18

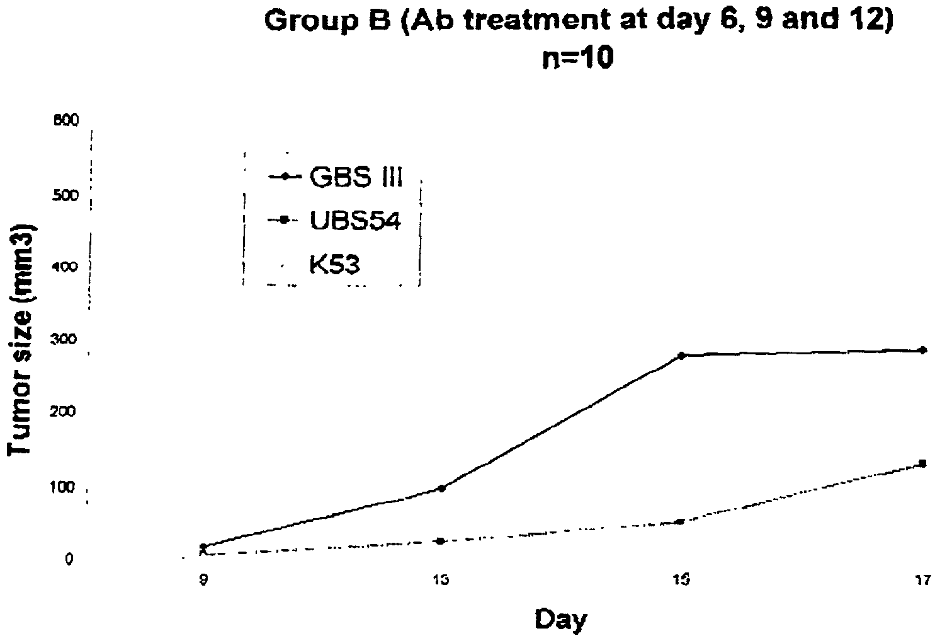


Figure 19

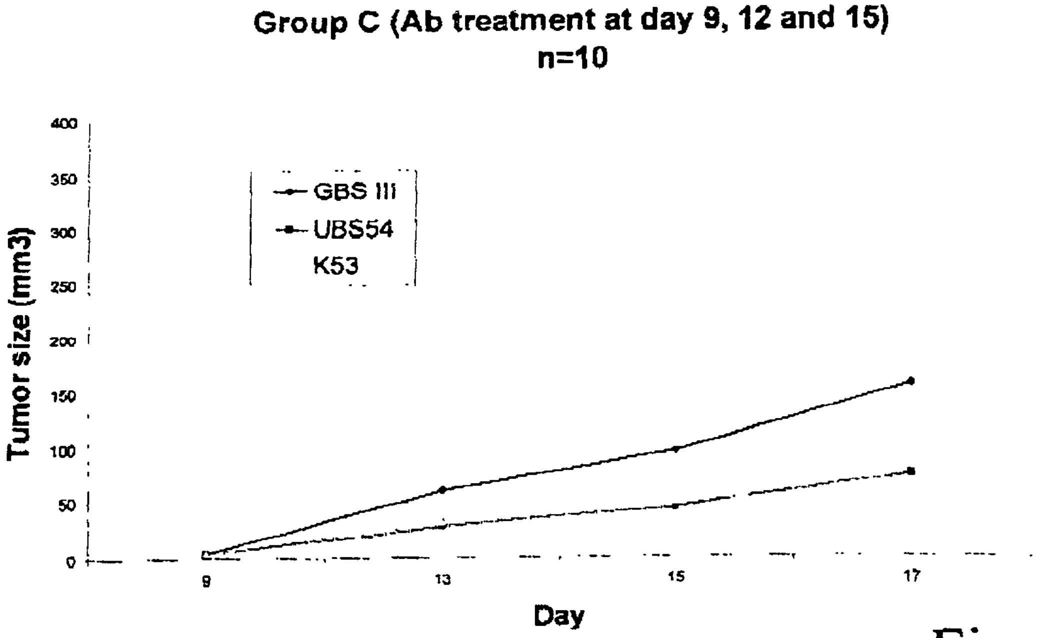


Figure 20

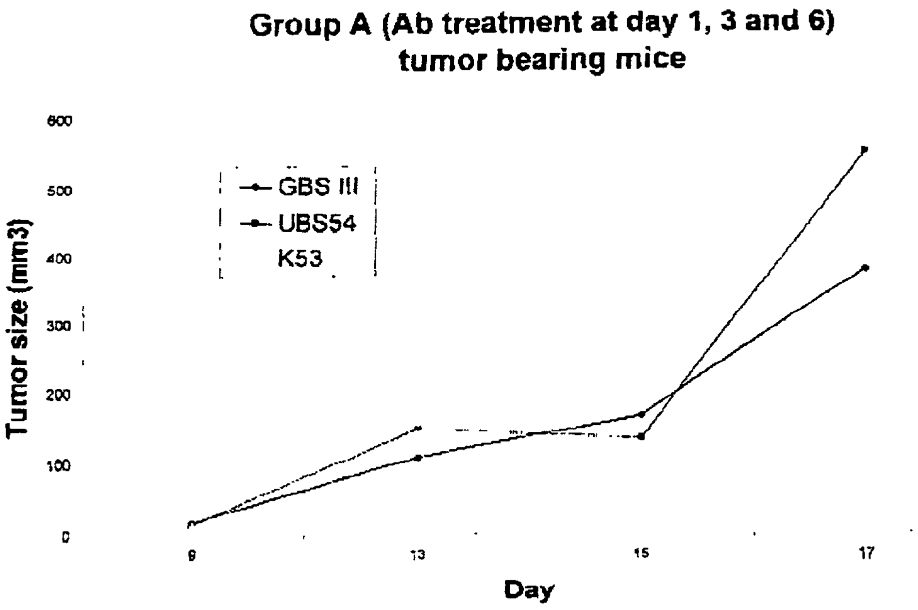


Figure 21

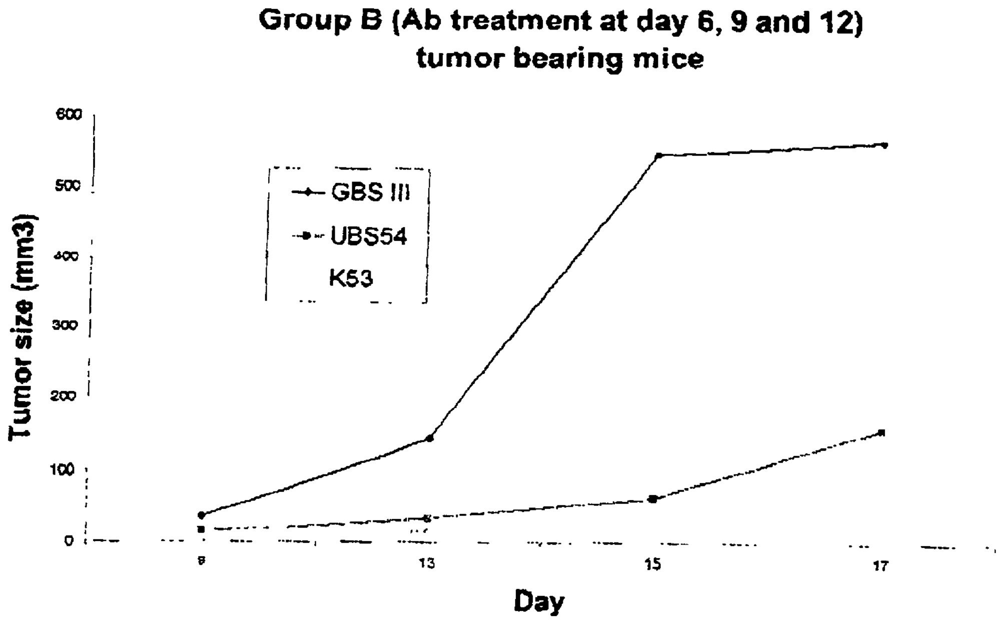


Figure 22

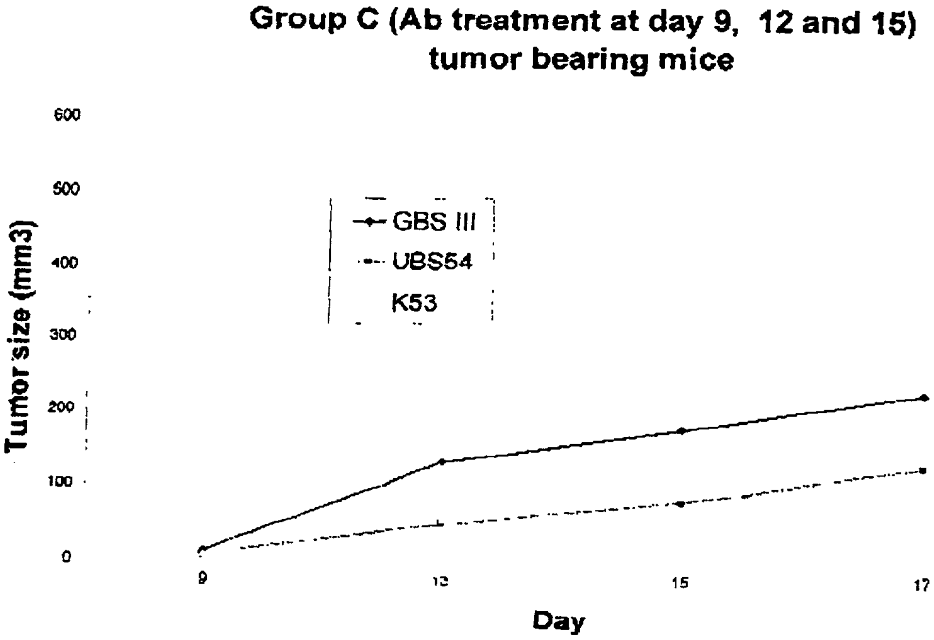


Figure 23

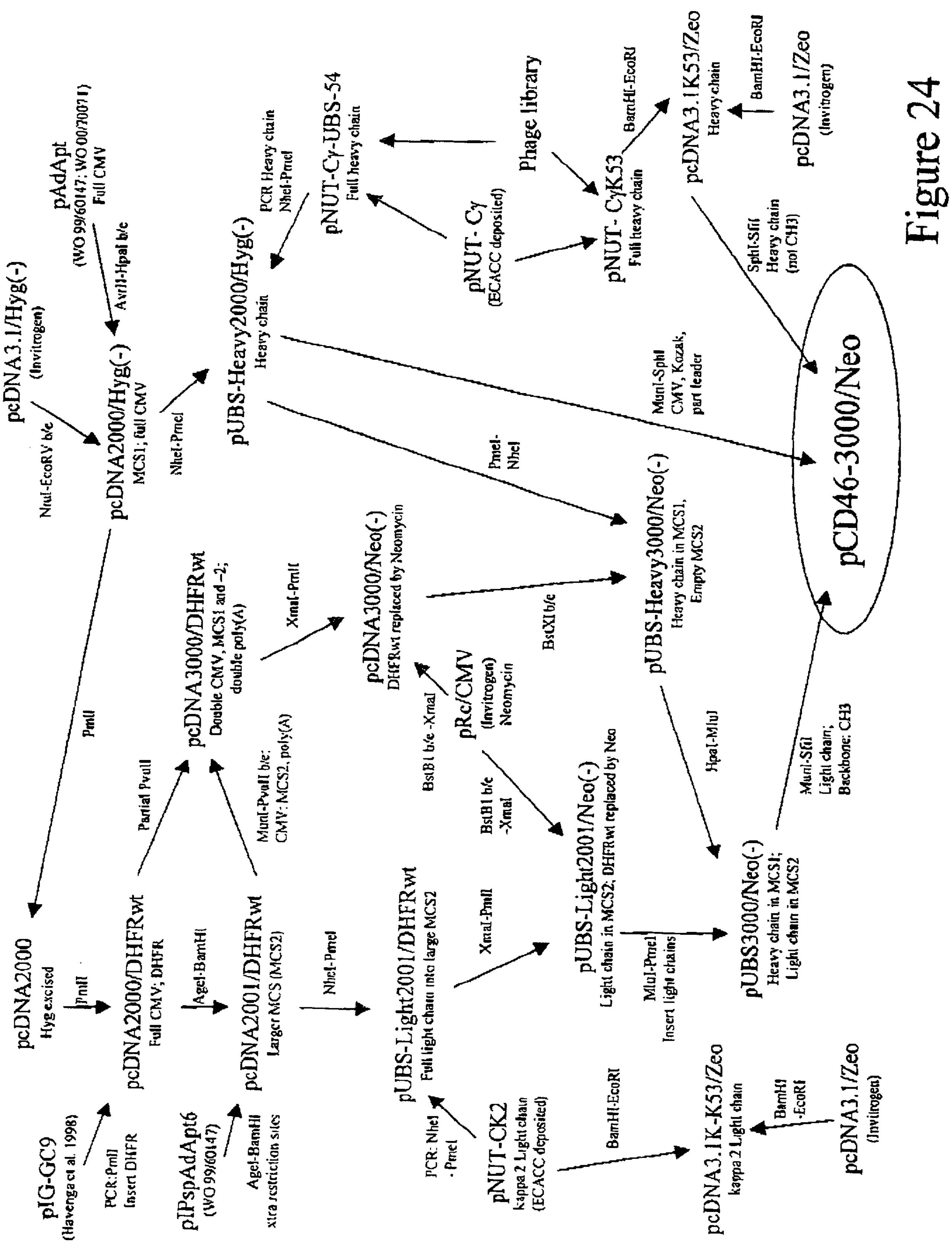

pcDNA3.1/Hyg(-)
(Invitrogen)
pAdApt
(WO 99/60147; WO 00/70071)
Full CMV
NruI-EcoRV b/e
AvrII-HpaI b/e
pcDNA2000/Hyg(-)
MCS1; full CMV
NheI-PmeI
pUBS-Heavy2000/Hyg(-)
Heavy chain
PCR Heavy chain
NheI-PmeI
pNUT-Cγ-UBS-54
Full heavy chain
Phage library
pNUT- Cγ
(ECACC deposited)
pNUT- CγK53
Full heavy chain
BamHI-EcoRI
pcDNA3.1K53/Zeo
Heavy chain
BamHI-EcoRI
pcDNA3.1/Zeo
(Invitrogen)
SphI-SfiI
Heavy chain
(not CH3)
MunI-SphI
CMV, Kozak,
part leader
pCD46-3000/Neo
PmeI-NheI
PmlI
pcDNA3000/DHFRwt
Double CMV, MCS1 and -2;
double poly(A)
XmaI-PmlI
pcDNA3000/Neo(-)
DHFRwt replaced by Neomycin
BstXI b/e
pUBS-Heavy3000/Neo(-)
Heavy chain in MCS1,
Empty MCS2
Partial PvuII
MunI-PvuII b/e:
CMV; MCS2, poly(A)
BstBI b/e -XmaI
pRc/CMV
(Invitrogen)
Neomycin
BstBI b/e
-XmaI
pUBS-Light2001/Neo(-)
Light chain in MCS2; DHFRwt replaced by Neo
HpaI-MluI
MunI-SfiI
Light chain;
Backbone: CH3
pUBS3000/Neo(-)
Heavy chain in MCS1;
Light chain in MCS2
pcDNA2000
Hyg excised
PmlI
pcDNA2000/DHFRwt
Full CMV; DHFR
AgeI-BamHI
pcDNA2001/DHFRwt
Larger MCS (MCS2)
NheI-PmeI
pUBS-Light2001/DHFRwt
Full light chain into large MCS2
XmaI-PmlI
MluI-PmeI
Insert light chains
pIG-GC9
(Havenga et al. 1998)
PCR:PmlI
Insert DHFR
pIPspAdApt6
(WO 99/60147)
AgeI-BamHI
xtra restriction sites
PCR: NheI
-PmeI
pNUT-CK2
Kappa 2 Light chain
(ECACC deposited)
BamHI-EcoRI
pcDNA3.1K-K53/Zeo
kappa 2 Light chain
BamHI
-EcoRI
pcDNA3.1/Zeo
(Invitrogen)

Figure 24

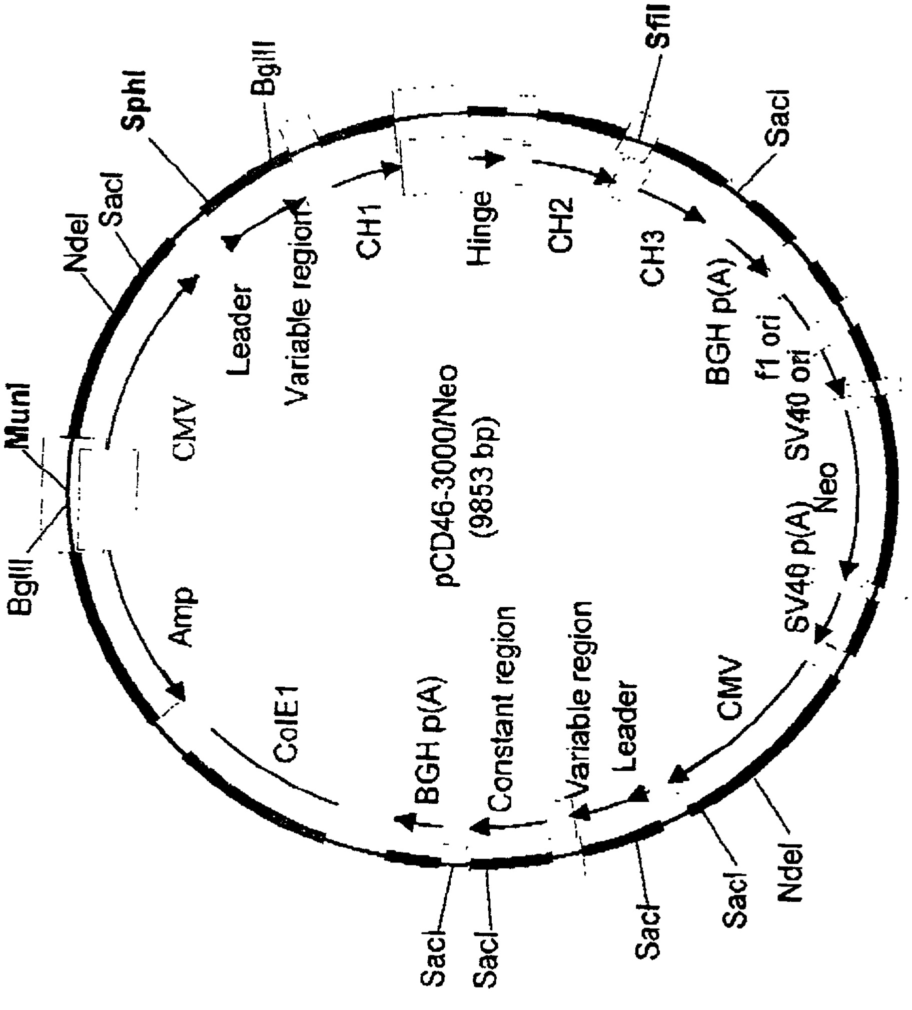

pCD46-3000/Neo
(9853 bp)
BglII
MunI
NdeI
SacI
SphI
BglII
SfiI
SacI
CMV
Leader
Variable region
CH1
Hinge
CH2
CH3
BGH p(A)
f1 ori
SV40 ori
Neo
SV40 p(A)
CMV
Leader
Variable region
Constant region
BGH p(A)
ColE1
Amp
NdeI
SacI
SacI
SacI
SacI

Figure 25

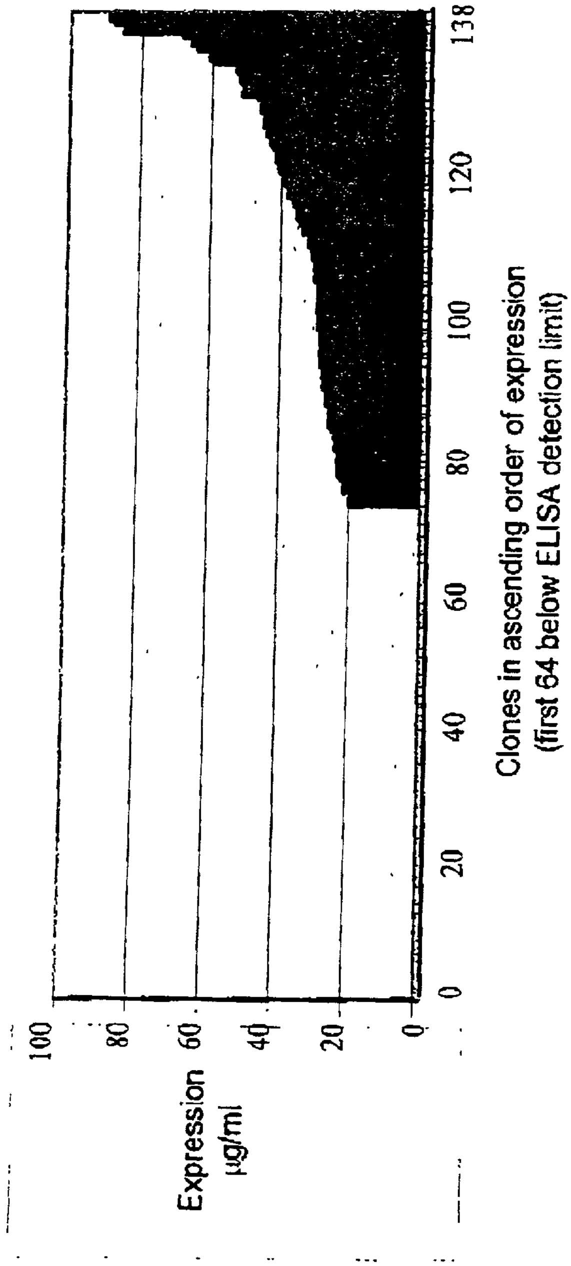

Expression
µg/ml
100
80
60
40
20
0
0
20
40
60
80
100
120
138
Clones in ascending order of expression
(first 64 below ELISA detection limit)

Figure 26

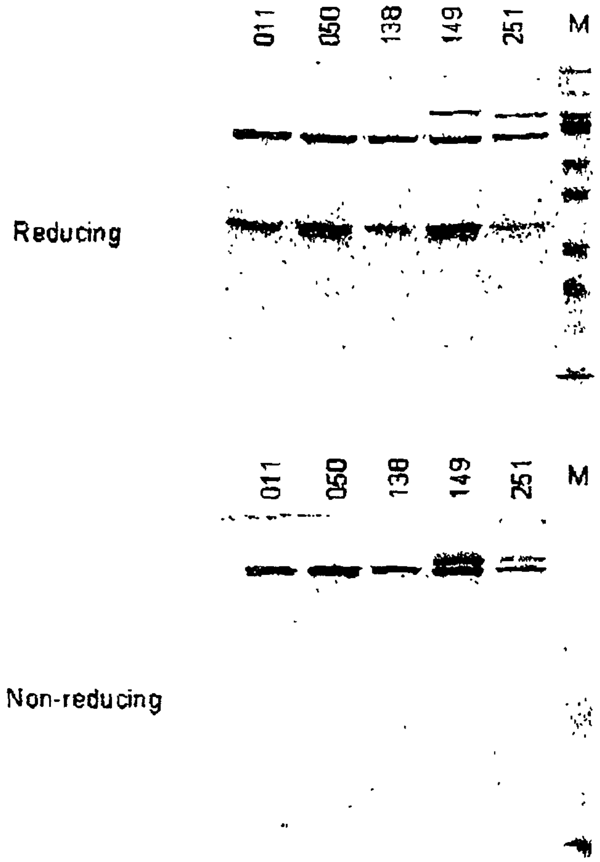

011
050
138
149
251
M
Reducing
011
050
138
149
251
M
Non-reducing

Figure 27

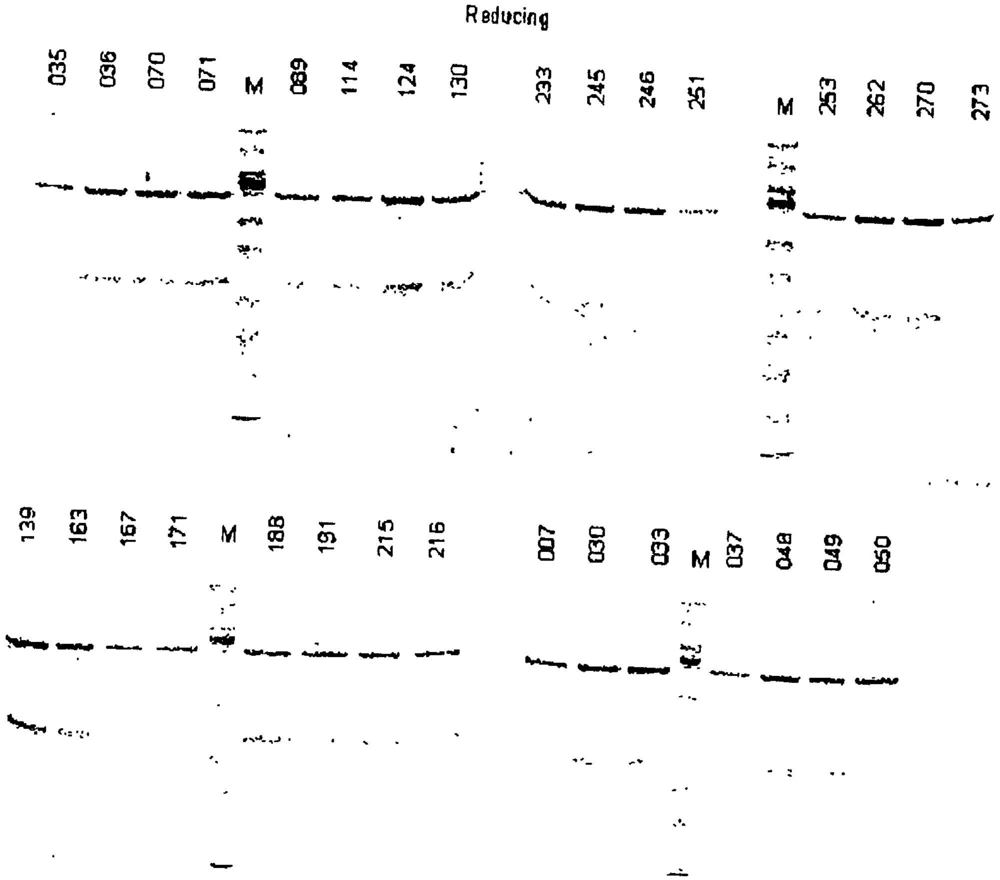

Reducing
035
036
070
071
M
089
114
124
130
233
245
246
251
M
253
262
270
273
139
163
167
171
M
188
191
215
216
007
030
033
M
037
048
049
050

Figure 28